RESEARCH PAPER | Zoology | Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

Angiogenic Activity of Hydroalcoholic Extract of Sea Urchin, *Temnopleurus Alexandri* (Bell, 1884)

KEYWORDS: Angiogenesis, CAM, GC-MS , Sea urchin

Parvathavarthini. R
Research and Development Centre, Bharathiar University, Coimbatore, Tamil Nadu, India.

Dr. Uma. B
Department of Zoology, Bharathi Women's College, Chennai,Tamil Nadu, India.

ABSTRACT *The present study eludicates the angiogenic activity of hydro alcoholic extract of the sea urchin, Temnopleurus alexandri (Bell, 1884). Marine organisms represent excellent source of bioactive compounds. The sea urchins were collected from Chennai coast and identified at ZSI, Chennai,Tamil Nadu, India . Hydro alcoholic extract was prepared by cold percolation method and its angiogenic activity was tested with CAM assay with different concentrations viz 10, 50, 100, 200, 300 and 500ng. GC-MS analysis was done. Values obtained and subjected to one way ANOVA and Mann-Whitney test. Further fractionation, purification and identification of the exact bio active molecule present in the Hydro alcoholic extract are of much importance and relevance in the current medical scenario.*

Introduction:
Natural products (NPs) traditionally have played an important role in drug discovery and were the basis of most early medicines (Newman et *al.*, 2000). This is especially the case in therapeutic areas such as oncology, immuno-suppression and metabolic diseases where NPs have played a central role in drug discovery. Angiogenesis or Neovascularization is a complex developmental process in which new blood vessels emerge from the pre – existing vasculature (Risau, 1997). Some of the new blood vessel formation is normal and beneficial. It is essential for a variety of physiological processes, including organ development, wound healing and reproduction, ischemic tissue restoration, restoring blood flow to tissue after injury or insult. Dysregulated angiogenesis is considered a common denominator in most frequent diseases including cancer, ischemic heart disease, blindness, psoriasis and arthritis (Carmeliet, 2005). A great deal of effort is now being devoted to the development of new drugs that hopefully control angiogenesis. The present study is aimed at assessing the angiogenic activity of hydro alcoholic extract of the sea urchin *T. alexandri* (Bell, 1884).

Materials and Methods:
Collection:
Freshly available sea urchin *T. alexandri* were collected from Kasimedu fish landing centre, Chennai coast during the month of July and August 2011. The Latitude and Longitude of Kasimedu is 13.125 and 80.295 respectively. Authentication of the echinoid was done with Zoological Survey of India (ZSI), Chennai (India). The taxonomic classification of the specimen used is as follows

§ Phylum : Echinodermata

§ Class : Echinoidea

§ Genus : Temnopleurus

§ Species : Alexandri

§ Authority : Bell,1884

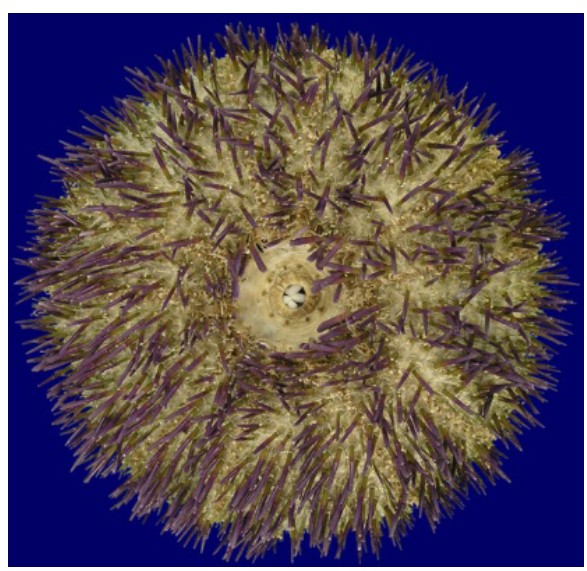


Fig.1: *Temnopleurus alexandri* **(Bell, 1884)**

Extraction:
Extraction was done using cold percolation method. 1 kg of specimen was shade dried at room temperature and ground with a manual mill. The powder (250g) was immersed in 750 ml of alcohol: water (80:20) (1:3 w/v) in an aspirator bottle for a period of 48 hours with occasional shaking. The extract was filtered through a Buchnner funnel with Whatmann No.1 filter paper. The extract was concentrated by removing the ethanol under reduced pressure using rotary evaporator at 40° C and the water was allowed to dry using the water bath. Finally crude extract was obtained. The crude extract was stored at 4° C until further use.

Chorio allontoic membrane (CAM) assay:
Angiogenic activity of hydro alcoholic extract was determined using the chick chorio allontoic membrane (Indap & Pathare, 2003). In brief, a window of 1cm^2 size was made in 5 days old embryonated eggs to observe CAM. Sterile antibiotic empty discs (Sigma) impregnated with known concentrations (10, 50, 100, 200 and 300ng/discs of 10µl) of the extract dissolved in 2% DMSO were placed on the CAM away from the central blood vessel. Eggs were further incubated for 48 hours at 37°C and the number of blood vessels were counted and

Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

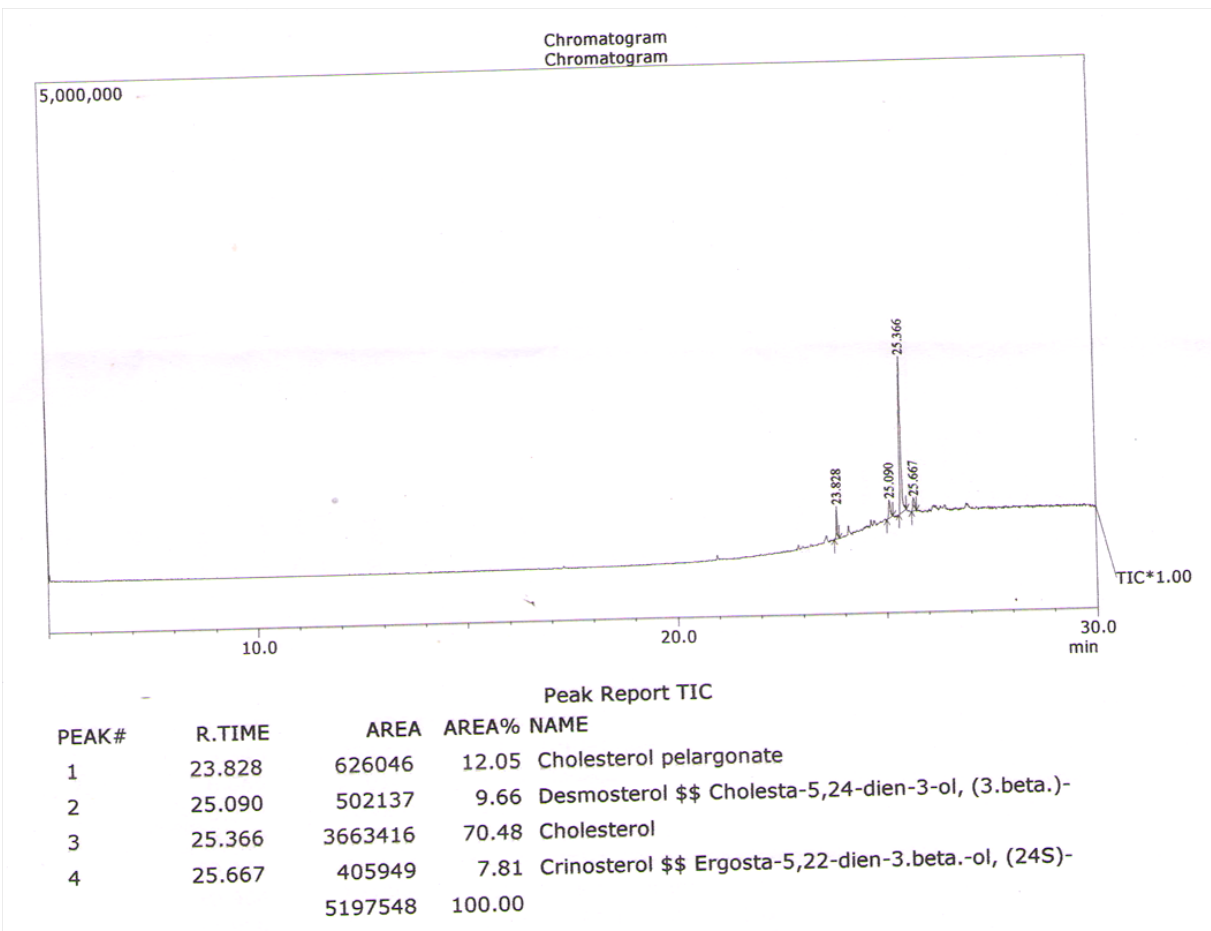
tabulated. Embryos treated with VEGF (100ng) and DMSO (2%) was used as positive and solvent control respectively.

Gas Chromatography – Mass spectrometry (GC-MS) Analysis:
The crude extract was identified and quantified using gas chromatograph (GCMS- Schimadzu) equipped with a DB-5 MS column (mm inner diameter 0.25 mm, length 30.0m, film thickness 0.25µm) mass spectrometer (ion source 200 °C, RI70eV) programmed at 40-650°C with a rate of 4°C/min. Injector temperature was 280°C; carrier gas was Helium (20psi), column flow rate was 1.4ml/min, injection mode – spirit.

Results:
The yield of extract was 1.05g. The result shows promising angiogenic activity. GC-MS analysis revealed presence of sterols like cholesterol pelargonate, Desmosterol, Cholesterol, Crinosterol and the same has to be confirmed with further studies.

Angiogenic activity:
The angiogenic activity of hydro alcoholic extract was found to be more effective at 300ng (190.33±8.144). The blood vessel count at different concentrations was 142.67±14.57 at 200ng, 79±3 at 100ng and 56±3 at 10ng. Among these the values were found statistically significant (p value <0.05) at 200 and 300ng. The solvent (DMSO) control used didn't interfere with the angiogenic activity since the number of blood vessels observed was same as observed with the control.

Fig.2: GC-MS report of Hydroalcoholic extract



Peak Report TIC

PEAK#	R.TIME	AREA	AREA%	NAME
1	23.828	626046	12.05	Cholesterol pelargonate
2	25.090	502137	9.66	Desmosterol $$ Cholesta-5,24-dien-3-ol, (3.beta.)-
3	25.366	3663416	70.48	Cholesterol
4	25.667	405949	7.81	Crinosterol $$ Ergosta-5,22-dien-3.beta.-ol, (24S)-
		5197548	100.00	

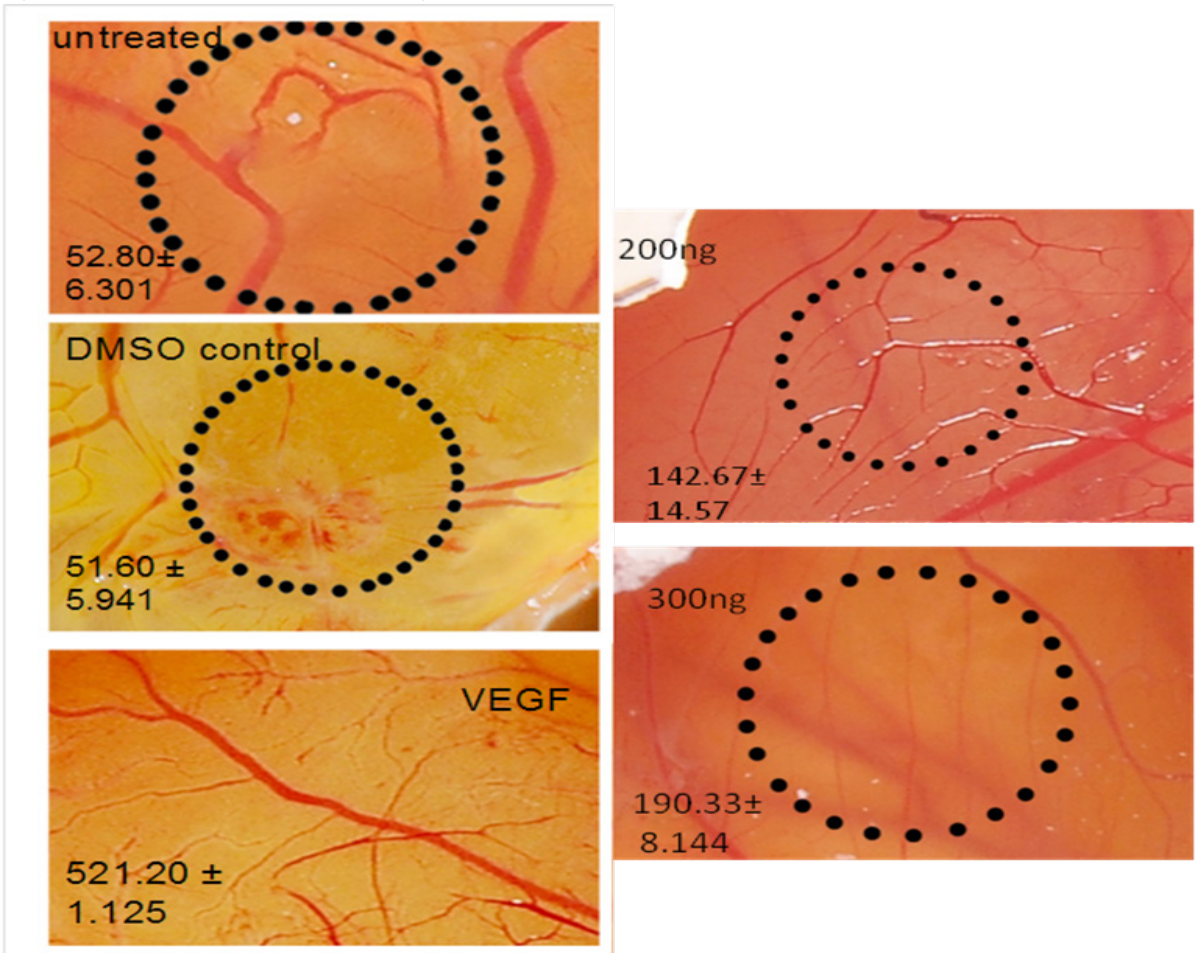
Table 1: Angiogenic activity – blood vessel count (mean of 5 values)

Untreated	DMSO	VEGF	10ng	100ng	200ng	300ng
52.80± 6.301	51.60± 5.941	521.20± 1.125	56± 3	79± 3	*142.67± 14.57	*190.33± 8.144

P values significant at <0.05 level

Volume : 4 | Issue : 1 | Jan 2014 | ISSN - 2249-555X

Figure 3: Blood vessel counts in CAM assay



Discussion:

Bioactive compounds that possess angiogenic activity are of interest in the field of pharmacology. The Hydro alcoholic extract of *T. alexandri* showed promising angiogenic activity. The major components in the present hydro alcoholic extract could have been responsible for the angiogenic activity.

Poly unsaturated fatty acids (arachidonic acid, eicosapentaenoic acid, docosa hexaenoic acid) isolated from sea urchin *Stichopus chloronotus* promoted wound healing, hence proving those compounds to be angiogenic (Bordbar *et a .l,* 2011). Similarly in our study, the crude extract contains fatty acids (cholesterol pelargonate, Desmosterol, Cholesterol, Crinosterol) which could be responsible for the angiogenic activity. Previously desmosterol that was isolated from a red sea weed *Galaxaura marginata* present significant cytotoxic and antibacterial activity (Sheu *et al .*, 1996) Also, anti inflammatory activity was observed with the oxygenated desmosterol that was isolated from a red sea weed *Galaxaura marginata* (Rozas & Freitas, 2012). One characteristic of crude extract is that its constituents may have opposite, moderating or enhancing effects. That is why the final activity depends on the interactions among the constituents and the effect of each constituent on its own (Gupta *et al.*, 2008). Hence , further fractionation, purification, and identification of the exact bioactive compound present in the crude extract is of much importance.

Acknowledgement

Dr.B.Uma wish to acknowledge the financial assistance rendered by UGC, New Delhi

REFERENCE

• Bordhar S, Anwar F and Saari N. (2011). High value components and bioactives from sea cucumber for functional foods-A review. Marine drugs. Vol.9, 1761-1805. | • Carmeliet P. (2005). Angiogenesis in life, disease and medicine. Nature, 438, 932-936. | • Gupta A, Upadhayay NK, Sawhney RC and Kumar R. (2008). A poly herbal formulation accelerates normal and impaired diabetic wound healing. Wound Rep Regen, 16, 784-790. | • Indap MM and Pathare SP. (2003). Fatty acids derived from a marine crustacean Diogenus avanus (Heller) & their anti-angiogenic activity. Indian J Exp Sci, 41, 632-635. | • Newman DJ, Cragg GM and Snader KM. (2000). Nat Prod Rep, 17, 215-234. | • Risau W. (1997). Mechanisms of angiogenesis. Nature (Lond), 386, pp: 671-674. | • Rozas EE and Freital JC. (2003). Antibacterial activity of the extract of the sea weed Galaxaura marginata (Rhodophyta, Nemaliales). J Venom Anim. Toxins incl. Trop Dis, 497. | • Sheu J, Huang S, Duh CH. (1996) Cytotoxic originated desmosterols of the red alga Galaxaura marginata. J Nat Prod, 59, 23-26. |